High Frequency of Detection of Epidermodysplasia Verruciformis–Associated Human Papillomavirus DNA in Biopsies from Malignant and Premalignant Skin Lesions from Renal Transplant Recipients

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Based on immunologic and epidemiologic data, it is plausible that skin cancer in renal transplant recipients is associated with human papillomaviruses (HPV). At present, conflicting evidence exists concerning the presence of HPV DNA in these cancers. We recently described a nested polymerase chain reaction method that enables the detection of all previously isolated epidermodysplasia verruciformis (EV)–associated HPVs. We now describe the detection of EV-associated HPV DNA in 49 (80%) of 61 biopsies from squamous cell carcinomas, in four (50%) of eight basal cell carcinomas, in 14 (93%) of 15 actinic keratoses, in two (40%) of five cases of Bowen's disease, and in four (57%) of seven keratoacanthomas.

HPV DNA typing revealed that all detected HPV types belonged to the EV-associated HPV types. A wide spectrum of EV-associated HPVs was found, including six putative new HPV types. In a high percentage of the lesions more than one HPV type was detected. We often found the same HPV types in different skin biopsies from both malignant and premalignant lesions from the same patient.

The high frequency of detection of EV-associated HPV types in biopsies from malignant and premalignant lesions is in agreement with the hypothesis that EV-associated HPVs are involved in the pathogenesis of skin cancer in renal transplant recipients. ***Key words: polymerase chain reaction/skin cancer. J Invest Dermatol 105:367–371, 1995***

Renal transplant recipients are at an increased risk for both warts and skin cancer [1–3]. Epidemiologic data suggest an important role for human papillomavirus (HPV) infection in the development of skin cancer in immunosuppressed patients. The number of keratotic skin lesions, consisting of warts, solar keratoses, and hyperkeratotic papillomas, is highly associated with both squamous cell carcinomas and basal cell carcinomas in renal transplant recipients [3,4]. In addition, patients without an apparent class switch from IgM to IgG to one of the capsid proteins (L1) of HPV type 8 appeared to have an increased risk of skin cancer as compared to patients with a good humoral response to this antigen [5]. HPV 8 belongs to the subgroup of HPV types that is predominantly associated with the rare hereditary disease epidermodysplasia verruciformis (EV), a disease characterized by extensive HPV-associated wart-like and scaly lesions and a high risk for the development of squamous cell carcinomas [6]. In the above-mentioned serologic tests cross-reactivity was noted between the HPV 8 L1 protein and L1 proteins of other EV-associated HPVs, but not with non–EV-associated HPVs like HPV 1, 2, 6, 16, or 18 [5]. Therefore, the serologic findings in renal transplant recipients suggest the presence of a virus belonging to the EV-associated subgroup rather than of HPV 8 alone.

The data concerning the detection of HPV DNA in skin lesions of renal transplant recipients are conflicting. Some authors have found a high prevalence of HPV DNA in skin carcinomas of these patients. Using dot blot hybridization, Barr *et al* [7] found DNA of the EV-associated HPV types 5 and 8 in 60% of 25 squamous cell carcinomas. The group from Lyon [8] found HPVs 1, 2, 16, and 18 in 47% of 30 squamous cell carcinomas using *in situ* hybridization, and the same group [9] reported mucosal HPV 6 and 11 in 71% of 24 squamous cell carcinomas using polymerase chain reaction (PCR) and additionally *in situ* hybridization and Southern blot hybridization. Recently Shamanin *et al* [10] reported the detection of a series of different HPV types (e.g., a novel HPV 29–related type) in benign and malignant skin lesions of renal transplant patients using a PCR with degenerate primers. Other groups found no HPV at all [11,12]. The explanation for this phenomenon of conflicting results is probably largely attributable to differences in the techniques used. For a meaningful assessment of the presence of HPV in these lesions, it is necessary to use a technique that is not only sensitive (for example, the PCR), but also able to detect a wide range of HPV types. In addition, the technique should be suitable for reliable typing of the HPV DNA.

Previously, we were able to detect DNA of EV-associated HPV

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Abbreviation: EV, epidermodysplasia verruciformis.

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in five (21%) of 24 squamous cell carcinomas of renal transplant recipients with four different PCR methods each using a different consensus primer pair (termed A, B, C, and D) located in the E1 open reading frame [13]. For the current study, a nested PCR mediated by four consensus primers was used with annealing sites in the L1 open reading frame of the viral genome. This PCR enabled the detection of all previously isolated EV-associated HPVs [14].

This study, utilizing this nested PCR, reports the detection of DNA of EV-associated HPV types in a high percentage of squamous cell carcinomas, basal cell carcinomas, and premalignant lesions from renal transplant recipients.

MATERIALS AND METHODS

Biopsy Specimens Ninety-six biopsy specimens were obtained from premalignant and malignant skin lesions from 44 renal transplant recipients. The specimens were snap-frozen and stored at −70°C. From each lesion a duplicate punch biopsy was taken for histologic examination. Control specimens consisted of 23 snap-frozen biopsies of non-tumorous skin lesions of 20 non-immunocompromised patients (vasculitis, chronic discoid lupus erythematosus, bullous pemphigoid, eczema, polymorphic light eruption, lichen planus, and non-specified dermatitis) and three biopsies of clinically normal skin from the buttocks of three renal transplant recipients.

DNA Preparation The snap-frozen skin biopsies were thoroughly minced, and cellular DNA was isolated by overnight incubation at 56°C in a 50 mM Tris-HCl (pH 8.5), 50 mM ethylenediaminetetraacetic acid solution containing 100 μg/ml proteinase K. Proteinase K was heat inactivated and DNA was subsequently extracted using the guanidinium isothiocyanate (GTC)–diatom method according to Boom and co-workers [15].

PCR Primers We used a nested PCR method as described previously [14] consisting of two degenerate primers, termed CP65 and CP70, located in the L1 open reading frame. The CP65/CP70 primer set (F) amplifies a 452–467-bp product depending on the target HPV type. For the second step (nested) PCR a primer set (G) consisting of the degenerate primers CP66 and CP69 was used, amplifying a 374–389-bp product. In a separate reaction, a β-globin PCR was carried out using the primer pair PCO3 (glo-1) and RS42 [16].

HPV Detection PCR amplification was performed exactly as previously described [14] using a 50-μl reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 3.6 mM $MgCl_2$, 0.1 mg/ml bovine serum albumin, 0.2 mM of each deoxynucleotide triphosphate, 1 U of Taq DNA polymerase, and 300 ng of both primers. In the first-step PCR, 5 μl of DNA (0.5 to 2% of total extractable DNA of the lesion) was used as an input. The first PCR amplification consisted of five cycles with a 1-min denaturation step at 95°C, a 1.5-min annealing step at 50°C, and a 2-min extension step at 72°C, followed by 35 cycles with a higher annealing temperature (1 min at 95°C, 1 min at 55°C, and 2 min at 72°C). In the second step (nested) PCR, 3 μl of the first step PCR was used as an input. In this PCR 30 cycles of amplification (1 min at 95°C, 1 min at 55°C, and 2 min at 72°C) were performed. For every two PCR reactions a negative control (water only) was included. These controls were processed the same way as the tissue specimens throughout the DNA preparation and the first- and second-step PCR and were never found positive for HPV.

With this technique all known EV-associated HPV genotypes could be amplified. The sensitivity of the PCR, as tested for four different EV-associated HPV types (HPV 8, 15, 20 and 23), ranged from 1 to 10 fg [14]. Other cutaneous HPV types, with the exception of HPV 1, could not be amplified or were only amplified by primer set F (HPV 2, 26, and 27). Genital HPV types 6, 11, 13, 16, 18, 31, and 33 could also be detected.

HPV DNA typing was performed by direct sequence analysis of the PCR amplimers as described earlier [14].

RESULTS

Ninety-six biopsies were taken from the following skin lesions from 44 renal transplant recipients: 61 squamous cell carcinomas, eight basal cell carcinomas, five cases of Bowen's disease, 15 actinic keratoses, and seven keratoacanthomas. The lesions were examined by a nested PCR method using first the consensus primer pair F followed by reamplification of the PCR product with the nested consensus primer pair G. The amplified HPV DNA fragments were typed by sequence analysis as described in *Materials and Methods*. The prevalence of HPV DNA is presented in **Table I**.

In 23 of the 55 HPV-positive skin lesions in which HPV typing was successfully performed by direct sequence analysis of the amplified fragment, more than one HPV type was present based on the sequence pattern. In most of these cases only the predominant HPV type could be determined. In only some of these cases we were able to molecularly clone and sequence the PCR amplimers, so that typing of the individual types in these HPV mixtures could be accomplished (see **Table I**). All samples that were sequenced contained DNA of HPV types that belong to the group of EV-associated HPV types or HPV types resembling these types. The following known HPV types were found: HPV 15, 19, 20, 21, 23, 24, 25, and 38. We often found genetic heterogeneity (intratypical variability) within the amplified sequences of these HPV types, especially among different patients. This intratypical variability averaged between 5 and 10%, but most of the nucleotide substitutions proved to be silent in the coding sequence. Phylogenetic analysis using the sequences of the PCR amplimers showed that the sequences of six HPV types (termed X1, X2, X3, X4, X5, and X6) were clearly distinct from the other known EV HPVs. The intertypical variability at nucleotide level ranged from 22 to 40%, indicating that X1 to X6 are putative novel HPV types belonging to the group of EV-associated viruses [14].

All control specimens, as described in *Materials and Methods*, remained negative, excluding contamination with HPV DNA and false positivity of the PCR. The DNA extracted from these control specimens was adequate for PCR analysis, because successful amplification of a β-globin PCR fragment was carried out in all cases.

In a previous study [13] we described the presence of EV-associated HPV types in five (21%) of 24 squamous cell carcinomas, in one of three cases of Bowen's disease, in two of seven actinic keratoses, and in one of five keratoacanthomas using four (single-step) PCR methods, each with a different consensus primer set (A, B, C, and D). For our present study we used the same biopsies as well as additional biopsy specimens. Comparing the results from our previous study with our nested PCR method (utilizing primer pairs F and G), it is clear that a much higher percentage of the samples turned out to be HPV positive (Table I). In addition, in five cases other HPV types were detected with our previous PCR method [13] as compared to the nested PCR. HPV 10 and an HPV

Table I. Prevalence of HPV DNA as Detected by Two Different PCR Methods in 96 Skin Lesions from 44 Renal Transplant Recipients

Type of Lesions	Number of Lesions	Number of Positive Lesions	HPV Types[a]
Squamous cell carcinoma	61	49 (80%)	$15^{(6x)}$, $M(15 + 20)^{2x}$, M(15 + X1), 19, $20^{(2x)}$, 21, $23^{(3x)}$, 24, $25^{(2x)}$, 38, $X1^{(3x)}$, X2, M(X2 + mix), X3, X4, $X5^{(2x)}$, X6, $mix^{(7x)}$, $ND^{(12x)}$
Basal cell carcinoma	8	4 (50%)	24, X1, $ND^{(2x)}$
Bowen's disease	5	2 (40%)	M(15 + 20), X4
Actinic keratosis	15	14 (93%)	$15^{(2x)}$, 24, M(X1 + mix), M(X4 + mix), $mix^{(5x)}$, $ND^{(4x)}$
Keratoacanthoma	7	4 (57%)	$M(15 + X1)^{(2x)}$, M(15 + mix), mix

[a] The HPV types were determined after direct sequencing of the PCR amplimer and by comparing this sequence with the sequences of the amplified PCR products of all known EV-associated HPV types. Some HPV types were found more then once, as indicated in superscript between parentheses. HPV X1 through X6 indicate novel, EV-associated HPV types (see [14]). M indicates the presence of more than one HPV type in one lesion, from which at least one type could be sequenced, as indicated between brackets. mix indicates the presence of more than one HPV type in one lesion, in which HPV typing was not successfully done. ND, not determined.

Table II. HPV Types Detected in Different Skin Lesions from a Selection of Renal Transplant Recipients with Three or More Lesions Studied

Patient Number	Sex/Age	Lesion[a]	Localization	Nested PCR	HPV Types[b]
1	F/41	SCC	Hand	+	24
		BCC	Upper arm	+	24
		Bowen's disease	Back	−	
2	M/52	SCC	Lower arm	+	X1
		SCC	Hand	+	X1
		SCC	Back	+	X1
		BCC	Face	+	X1
3	M/47	SCC	Hand	+	25
		SCC	Hand	+	20
		SCC	Elbow	+	25
4	M/46	SCC	Face/skull	+	Mix
		SCC	Face/skull	+	23
		SCC	Wrist	−	
5	F/65	SCC	Finger	+	Mix
		SCC	Lower arm	+	X4
		SCC	Lower leg	+	38
		SCC	Lower leg	+	ND
		Bowen's disease	Upper arm	+	X4
6	M/63	SCC	Hand	+	ND
		SCC	Hand	+	Mix
		SCC	Face	+	M(15 + X1)
		SCC	Hand	+	15
		Actinic keratosis	Face	+	Mix
		Actinic keratosis	Face	+	Mix
		Keratoacanthoma	Face	+	M(15 + X1)
		Keratoacanthoma	Face	+	M(15 + X1)
7	M/41	SCC	Lower leg	−	
		SCC	Lower leg	+	ND
		SCC	Hand	+	Mix
		Actinic keratosis	Upper leg	+	15
8	M/68	SCC	Lower leg	+	M(15 + 20)
		SCC	Face	+	M(15 + 20)
		SCC	Hand	+	Mix
		Bowen's disease	Face	+	M(15 + 20)

[a] SCC, squamous cell carcinoma; BCC, basal cell carcinoma.
[b] HPV X1 and X4 indicate novel HPV types. M indicates the presence of more than one HPV type in one lesion, from which at least one type could be sequenced, as indicated between brackets. Mix indicates the presence of more than one HPV type in one lesion, in which HPV typing was not successfully done. ND, not determined.

10–related HPV type were found in one actinic keratosis and in one keratoacanthoma with primer set A, whereas with the nested PCR a mixture of yet untyped HPVs and no HPV was detected respectively. In one case of Bowen's disease HPV 36 was found with our previous PCR method, whereas the nested PCR revealed the presence of HPV 15 and 20. In two squamous cell carcinomas HPV 14 and an HPV 14–related HPV type were found with our previous PCR method, and a yet untyped mixture of HPV types was found with the nested PCR. These apparent discrepant results are probably due to the relatively frequent occurrence of mixed HPV infections in the skin lesions in combination with the different spectrum of HPV types detected by the different PCR methods.

In **Table II** the PCR results of eight patients of whom three or more skin lesions were studied are presented. The same HPV types were often detected in different biopsies from skin lesions from the same patient. It is very unlikely that this is due to carryover of HPV DNA from one specimen to another, because most skin biopsies from different lesions from the same patient were obtained and processed on different dates. Moreover, negative controls processed in between the patient samples remained negative. Of course, we cannot exclude the possibility that the patients themselves are the source of contamination, spreading HPV from one lesion to another.

The results of nested PCR amplification of HPV DNA present in biopsies from skin lesions from four renal transplant recipients are presented in **Fig 1**.

DISCUSSION

With our recently developed nested PCR method, we found HPV DNA in a high percentage of malignant and premalignant skin lesions from renal transplant recipients. In our single-step PCRs (primer sets A, B, C, and D) [13], we detected HPV DNA in no more than 21% of the skin cancers. In contrast, using the more sensitive nested PCR approach, we detected HPV DNA in 80% of the same skin cancers ([14] and **Table I**). The present data show that not only the malignant, but also a high percentage of other

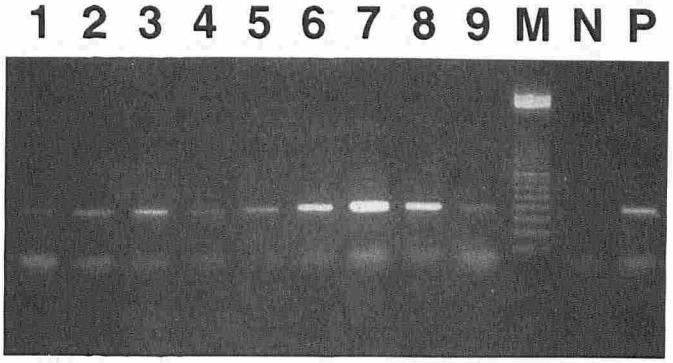

Figure 1. PCR products are shown after electrophoresis in an ethidium bromide–stained gel. Patient 1 in **Table II**, basal cell carcinoma (BCC) (*lane 1*) and squamous cell carcinoma (SCC) (*lane 2*); patient 2, SCC (*lane 3*), BCC (*lane 4*), and SCC (*lane 5*); patient 3, three SCCs (*lanes 6–8*); patient not mentioned in **Table II**, actinic keratosis (*lane 9*). A 100–base pair marker (*lane M*), a negative control (water) (*lane N*), and a positive control (HPV 8) (*P*) are included.

lesions (Bowen's disease, actinic keratoses and keratoacanthomas) of renal transplant recipients contain HPV DNA.

We detected HPV types that until now were presumed to be largely restricted to skin lesions from patients suffering from EV. In addition, we detected six putative new HPV types that were found to be closely related to the already known EV-associated HPV genotypes. For the definitive recognition of these types as novel types the sequences of the E6, E7, and L1 open reading frames of the cloned genomes must be compared with that of all other known HPV types.

Direct sequencing of the PCR product allows reliable determination of the HPV types (and subtypes) present [14]. Interestingly, using this method, we did not detect HPV 5 and 8 in any of the 96 tested skin lesions. These HPV types are the main HPV types described to be associated with skin cancers in EV patients [6]. HPV 5 was also the first HPV type detected in skin cancers of a renal transplant recipient by Southern blotting [17]. Moreover, a high prevalence of HPV 5 and 8 in malignant and premalignant skin lesions from renal transplant recipients was observed by the group from Edinburgh [7]. For their study a dot-blot hybridization technique was used, which is not only a less sensitive but also a less specific technique than the PCR. Therefore, it cannot be excluded that their biopsies contained other EV-associated HPV types instead of HPV 5 and 8 and that cross-hybridization caused a positive signal in their assay. In a recent publication, using type-specific PCR primers, the same group did not find HPV 5 and 8 DNA in 31 squamous cell carcinomas from renal transplant recipients. They detected (mostly non-typed) HPV DNA in 33% of the squamous cell carcinomas by Southern blot analysis and HPV 5 (but not HPV 8) DNA in only two of 50 premalignant lesions and three of 18 viral warts [18]. The group from Lyon did not detect HPV 5 DNA in a series of 27 malignant skin tumors from renal transplant recipients by *in situ* hybridization [19]. They found, however, that one of eight basal cell carcinomas and seven of 19 squamous cell carcinomas contained (unspecified) HPV DNA sequences other than HPV 5. Recently, Shamanin *et al* [10] reported the detection of HPV DNA in 11 of 20 squamous cell carcinomas, three of five basal cell carcinomas, and two of eight cases of Bowen's disease using PCR with degenerate primers. Most of the HPV types present in the (pre)malignant skin lesions were uncharacterized. In one of these samples, EV-associated HPV type 20 was identified, and in three SCCs HPV type 29–related DNA was found. In the other (pre)malignant skin lesions other known HPV types, especially cutaneous types, were identified. In some of the benign skin lesions EV-associated HPV type 12, 15, 17, and 25 were detected.

Comparing the results of the above-mentioned studies, one must realize that the use of different techniques could lead to the detection of different HPV types. Our data indicate that often more than one HPV type is present in a single skin biopsy from a renal transplant recipient. As mentioned before, we found a mixture of HPV types in 23 of 55 (42%) HPV-positive skin lesions, in which HPV typing was succesfully done by direct sequence analysis. It is therefore quite possible that different HPV types can be detected in the same lesion using PCR techniques with different specificities. This probably also explains the discrepant results obtained for five cases with our previous PCR method and the nested PCR method described in this manuscript.

We did not find mucosal HPV types 6 and 11 in any of the malignant or premalignant skin lesions, although our nested PCR method was able to detect HPV types 6 and 11. Therefore we have no good explanation for the high prevalence of these HPV types in skin lesions as reported recently by the group from Lyon [9]. HPV 6 and 11 are usually only detected in benign genital lesions such as condylomas or benign laryngeal papillomas. The skin lesions described by the group from Lyon were not checked for EV-associated HPVs other than HPV 5, so it cannot be excluded that these HPV types were present in their biopsies.

The presence of EV-associated HPV DNA in a high percentage of the biopsies from malignant and premalignant lesions in renal transplant recipients and the recurrent finding in several patients of the same HPV types give support to the hypothesis that these HPV types play a role in the development of skin cancer in renal transplant recipients. On the other hand, the presence of multiple HPV types in one skin biopsy might argue in favor of the presence of HPVs as "passengers" in these lesions, in which case they may not be involved in the pathogenesis of these lesions.

Some of the carcinomas remained negative with our nested PCR approach. It is possible that these carcinomas contained a very low copy number of EV-associated HPV genomes or that still other HPV types are present in these samples, which cannot efficiently be detected by our nested PCR. Another hypothesis is that HPV is only important for the initiation but not for the maintenance of the malignant phenotype ("hit-and-run" mechanism). In accordance with this hit-and-run hypothesis, Purdie *et al* [20] described that HPV DNA hybridizing with HPV 5/8 was detectable in two of three cell lines derived from skin cancers of renal transplant recipients, but only at early passages.

At this moment we can only speculate about the site of active virus replication. Neither in the study of Purdie *et al* [20] nor in our study was it possible to distinguish between HPV DNA contained within the malignant cells and HPV DNA derived from cells from the marginal (e.g., premalignant) tissue. An approach developed by our research group, analyzing alternating sections of paraffin-embedded tissue by means of histology and PCR [21], could solve this problem. Therefore, we are currently adapting our PCR technique for use on paraffin-embedded tissue.

Many additional questions remain to be solved before it is possible to estimate the significance of the apparent presence of EV-associated HPV DNA in biopsies of malignant and premalignant skin lesions in relation to the pathogenesis of skin cancer, such as whether these EV-associated HPV types are transcriptionally active in (pre)malignant skin lesions and, if so, which viral genes are transcribed.

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REFERENCES

1. Hartevelt MM, Bouwes Bavinck JN, Kootte AMM, Vermeer BJ, Vandenbroucke JP: Incidence of skin cancer after renal transplantation in the Netherlands. *Transplantation* 49:506–509, 1990
2. Boyle J, MacKie RM, Briggs JD, Junor BJR, Aitchison TC: Cancer, warts, and sunshine in renal transplant recipients: a case-control study. *Lancet* 1:702–705, 1984
3. Bouwes Bavinck JN, Vermeer BJ, van der Woude FJ, Vandenbroucke JP, Schreuder GMT, Thorogood J, Persijn GG, Claas FHJ: Relation between skin cancer and HLA antigens in renal-transplant recipients. *N Engl J Med* 325:843–848, 1991
4. Bouwes Bavinck JN, de Boer A, Vermeer BJ, Hartevelt MM, van der Woude FJ, Claas FHJ, Wolterbeek R, Vandenbroucke JP: Sunlight, keratotic skin lesions and skin cancer in renal transplant recipients. *Br J Dermatol* 129:242–249, 1993
5. Bouwes Bavinck JN, Gissmann L, Claas FHJ, van der Woude FJ, Persijn GG, ter Schegget J, Vermeer BJ, Jochmus I, Müller M, Steger G, Gebert S, Pfister H: Relation between skin cancer, humoral responses to human papillomaviruses, and HLA class II molecules in renal transplant recipients. *J Immunol* 151:1579–1586, 1993
6. Orth G: Epidermodysplasia verruciformis. In: Salzman NP, Howley PM (eds.). *The Papovaviridae, the Papillomaviruses*. Plenum Publishing Corp., New York, 1987, pp 199–243
7. Barr BBB, Benton EC, McLaren K, Bunney MH, Smith IW, Blessing K, Hunter JAA: Human papilloma virus infection and skin cancer in renal allograft recipients. *Lancet* 1:124–129, 1989
8. Euvrard S, Chardonnet Y, Pouteil-Noble C, Kanitakis J, Chignol MC, Thivolet J, Touraine JL: Association of skin malignancies with various and multiple carcinogenic and noncarcinogenic human papillomaviruses in renal transplant recipients. *Cancer* 72:198–206, 1993
9. Soler C, Chardonnet Y, Allibert P, Euvrard S, Schmitt D, Mandrand B: Detection of mucosal human papillomavirus types 6/11 in cutaneous lesions from transplant recipients. *J Invest Dermatol* 101:286–291, 1993
10. Shamanin V, Glover M, Rausch C, Proby C, Leigh IM, zur Hausen H, de Villiers E-M: Specific types of human papillomavirus found in benign proliferations

and carcinomas of the skin in immunosuppressed patients. *Cancer Res* 54:4610–4613, 1994

11. Dyall-Smith D, Trowell H, Mark A, Dyall-Smith M: Cutaneous squamous cell carcinomas and papillomaviruses in renal transplant recipients: a clinical and molecular biological study. *J Dermatol Sci* 2:139–146, 1991
12. Smith SE, Davis IC, Leshin B, Fleischer A, White WL, Feldman SR: Absence of human papillomavirus in squamous cell carcinomas of nongenital skin from immunocompromised renal transplant patients. *Arch Dermatol* 129:1585–1588, 1993
13. Tieben LM, Berkhout RJM, Smits HL, Bouwes Bavinck JN, Vermeer BJ, Bruijn JA, van der Woude FJ, ter Schegget J: Detection of epidermodysplasia verruciformis-like human papillomavirus types in malignant and premalignant skin lesions of renal transplant recipients. *Br J Dermatol* 131:226–230, 1994
14. Berkhout JM, Tieben LM, Smits HL, Bouwes Bavinck JN, Vermeer BJ, ter Schegget J: Detection and typing of epidermodysplasia verruciformis-associated human papillomavirus types in cutaneous cancers from renal transplant recipients: a nested PCR approach. *J Clin Microbiol* 33:690–695, 1995
15. Boom R, Sol CJA, Salimans MMM, Jansen CL, Wertheim-van Dillen PME, van der Noordaa J: Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* 28:495–503, 1990
16. Greer CE, Peterson SL, Kiviat NB, Manos MM: PCR amplification from paraffin-embedded tissues. Effects of fixative and fixation time. *Am J Clin Pathol* 95:117–124, 1991
17. Lutzner MA, Orth G, Dutronquay V, Ducasse MF, Kreis H, Crosnier J: Detection of human papillomavirus type 5 DNA in skin cancers of an immunosuppressed renal allograft recipient. *Lancet* II:422–424, 1983
18. Stark LA, Arends MJ, McLaren KM, Benton EC, Shahidullah H, Hunter JAA, Bird CC: Prevalence of human papillomavirus DNA in cutaneous neoplasms from renal allograft recipients supports a possible viral role in tumour promotion. *Br J Cancer* 69:222–229, 1994
19. Soler C, Chardonnet Y, Euvrard S, Chignol MC, Thivolet J: Evaluation of human papillomavirus type 5 on frozen sections of multiple lesions from transplant recipients with in situ hybridization and non-isotopic probes. *Dermatology* 184:248–253, 1992
20. Purdie KJ, Sexton CJ, Proby CM, Glover MT, Williams AT, Stables JM, Leigh IM: Malignant transformation of cutaneous lesions in renal allograft patients: a role of human papillomavirus. *Cancer Res* 53:5328–5333, 1993
21. Cornelissen MTE, van den Tweel JG, Struyk APMB, Jebbink MF, Briët M, van der Noordaa J, ter Schegget J: Localization of human papillomavirus type 16 DNA using the polymerase chain reaction in the cervix uteri of women with cervical intraepithelial neoplasia. *J Gen Virol* 70:2555–2562, 1989